P106- DIFFUSE LYMPHADENITIS AND DISSEMINATED *Mycobacterium avium* subsp. *paratuberculosis* INFECTION IN TWO WILD EURASIAN OTTERS (*Lutra lutra* L. 1758)

[1]Matos AC, [1]Figueira L, [1]Martins MH, [2]Matos M, [3]Pires MA, [1]Álvares S, [1]Mendes A, [1]Sousa N, [3]Coelho A, [3]Pinto ML

[1]Polytechnic Institute of Castelo Branco, Castelo Branco, Portugal
[2]Department of Genetics and Biotechnology, University of Trás-os-Montes e Alto Douro, Vila Real, Portugal
[3]Department of Veterinary Sciences and Centre for Studies on Agricultural and Veterinary Sciences, University of Trás-os-Montes e Alto Douro, Vila Real, Portugal

INTRODUCTION

Eurasian otters (*Lutra lutra*, L. 1758) are diving mammals of the *Mustelidae* family, order Carnivora that live almost exclusively in riparian habitats[1]. This species has a Palaearctic distribution and has suffered a significant decline in the last century which has led to local extinctions in many regions of Europe[2]. They can be carriers of mycobacteria, namely *Mycobacterium bovis*[3], but *Mycobacterium avium* subsp. *paratuberculosis* (Map) was never referred in these animals.

MATERIAL AND METHODS

Two Eurasian otters (*Lutra lutra*, L. 1758), one female and one male, were found dead due to vehicular trauma in Idanha-a-Nova, Portugal (39° 55' 11" North, 7° 14' 12" West) and submitted to necropsy. Samples consisting of liver, spleen, kidney, intestine and lymph nodes were collected for histopathology, bacteriological culture and polymerase chain reaction (PCR) analysis. For histopathologic studies, samples of all organs were fixed in 10% neutral buffered formalin, embedded in paraffin according to standard laboratory procedures, sectioned and stained with hematoxilin-eosin, Ziehl-Neelsen method for acid-fast bacilli, Perl's and Congo red stains. Deoxyribonucleic acid (DNA) was extracted from tissue samples for direct PCR using a commercial kit (Dneasy Blood & Tissue®, Quiagen, 40724 Hilden, Germany), and from colonies using a commercial kit (UltraClean® Microbial DNA Isolation, MO BIO Laboratories, Inc., 92010 Carlsbad, California), according to the manufacturer's instructions. To confirm the agent, IS900 PCR was performed using the method described by Garrido et al. (2000). Samples were decontaminated with 0.75% (w/v) hexadecyl pyridinium chloride (HPC; Sigma-Aldrich, Milano 20151, Italy) and cultured in duplicate onto slants of Löwenstein-Jensen medium® (Liofilchem, Roseto degli Abruzzi 64026, Italy) containing mycobactin J® (Synbiotics Europe SAS, Lyon 69367, France).

RESULTS

On gross examination, the organs showed no significant alterations, however, microscopically, the retropharyngeal and mesenteric lymph nodes of both otters presented disrupted architecture, lymphoid depletion and diffuse inflammatory infiltrate composed mainly of macrophages and, to a lesser extent, neutrophils (no granulomas or multinucleated giant cells observed) (Fig. 1A). The macrophages contained golden-brown pigment, resembling hemosiderin (Fig. 1B), but were negative to the Perl's reaction. A hyaline material, similar to amyloid, was observed in the center of the lymphoid follicles (Fig. 1C). Congo red staining for this material was inconclusive. The ileum, as well as the liver, spleen, lungs and kidneys had no microscopic alterations. The Ziehl-Neelsen method applied to the tissue sections did not reveal the presence o acid-fast bacilli but, Map was isolated from tissues collected from both otters. In the female, Map was detected by direct PCR in liver, spleen and mesenteric lymph node. In the male, Map was detected in mesenteric and retropharyngeal lymph nodes.

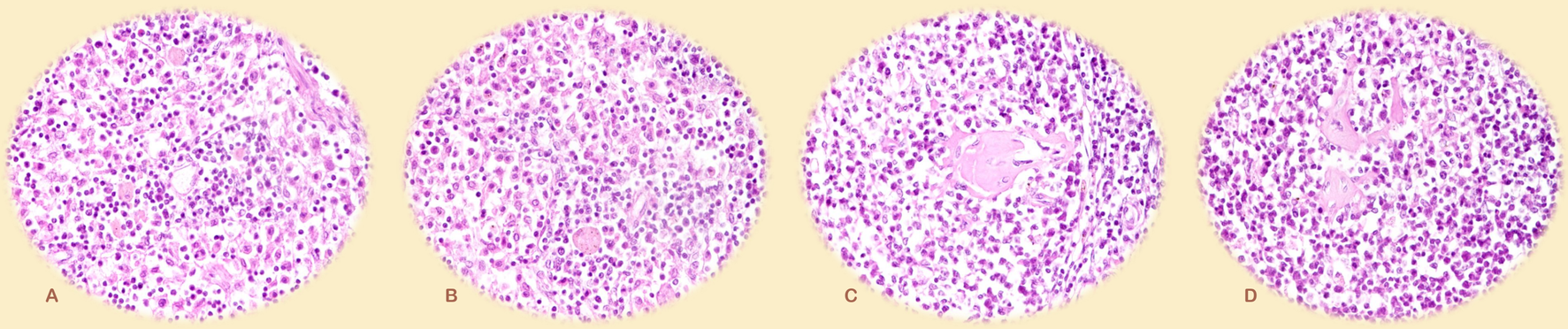

Figure 1: Morphological aspects of the diffuse lymphadenitis in the retropharyngeal and mesenteric lymph nodes of wild Eurasian otters (*Lutra lutra*, L. 1758) infected with Map: disrupted architecture, lymphoid depletion and diffuse inflammatory infiltrate composed mainly of macrophages, containing golden-brown pigment, resembling hemosiderin, and few neutrophils (A and B); Hyaline material, similar to amyloid, at the center of the lymphoid follicles (C and D). Haematoxylin and Eosin. Original magnification for all the images, 40x.

DISCUSSION AND CONCLUSION

The occurrence of paratuberculosis infection has been well documented in non-ruminant wildlife, but in wild carnivores the studies about Map infection are scarce[5]. While a study of wild carnivores reported 38% direct PCR positive results, only in one tissue was viable Map isolated[6]. A recent study in Southwestern Europe showed little or no evidence of Map infection in wild canids[7]. Herein we confirm that Eurasian otters can be a carrier of mycobacteria, specifically of Map, which, to the best of our knowledge, was never described before.

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